## *REMARKS*

Claims 73-144 are pending in the application. Claims 73-97, 103, 121 and 129-141 have been withdrawn from consideration. Claims 98-102, 104-120, 122-128 and 142-144 have been rejected.

Claims 98-102, 104-120, 122-128, 142 and 143 have been objected to. Claims 110-113, 127 and 143 have been rejected under 35 USC 112. Claims 98-102, 110-119, 127 and 128 have been rejected under 35 USC 102(b) as anticipated by Garger et al. Claims 104-107 and 122-125 have been rejected under 35 USC 103(a) as being obvious over Garger et al. in view of Boller et al. and Stomp et al. Claims 98-102, 104-113, 115-120, 122-128 and 142-144 have been provisionally rejected under 35 USC 101 for non-statutory double patenting as claiming the same invention as claims of co-pending US Application 11/790991. Claims 1-72, 99, 101-102, 104-105, 110-113, 116, 118-119 and 122-123 have now been canceled. Claims 98, 106-109, 114-115, 120, 124-127 and 142-143 have now been amended. New claims 145-149 have been added.

### *Specification*

1. The Examiner has objected to the Title of the invention as lacking adequate descriptiveness. The Title has been amended to recite: RECOMBINANT HIGH MANNOSE HUMAN LYSOSOMAL PROTEINS FROM PLANT CELL CULTURE

2. The Specification has been amended to include SEQ identifiers for the sequences referred to on pages 31 and 32.

3. SEQ ID NO: 8: The Examiner has correctly noted that the polypeptide depicted by SEQ ID NO: 8 lacks a significant portion of the human glucocerebrosidase polypeptide. Applicant acknowledges this error, and respectfully requests amendment of SEQ ID NO: 8 to include the entire amino acid sequence of human glucocerebrosidase as published (GLCM_HUMAN) prior to the filing of the present application.

Applicant submits that the amino acid sequence of the truncated SEQ ID NO: 8 is a mere typographical error, and does not represent the actual polypeptide sequence referred to as SEQ ID NO: 8 throughout the instant specification. Evidence for the unintentional and technical nature of this unfortunate mistake is provided herewith.

a. The amino acid structure of human glucocerebrosidase is clearly identified in the specification (see page 1, lines 20-28).

b. SEQ ID NO: 8 is clearly disclosed as the full length human glucocerebrosidase polypeptide, as encoded by the polynucleotide having nucleic acid sequence of SEQ ID NO: 7 (see page 14, lines 24-25). SEQ ID NO: 7 is the complete human glucocerebrosidase coding sequence, and is not missing the portion noted by the Examiner.

c. The claimed recombinant human lysosomal enzyme is further disclosed as the full length human glucocerebrosidase polypeptide, by reference to the polynucleotide of SEQ ID NO: 13 and the polypeptide of SEQ ID NO: 14, which include the amino acid sequence of the full length human glucocerebrosidase polypeptide as set forth in SEQ ID NO: 8:

> "Preferably, the GCD comprises the amino acid sequence substantially as denoted by SEQ ID NO: 8, encoded by the nucleic acid sequence as denoted by SEQ ID NO: 7.
> More preferably, the cell is transformed or transfected with a recombinant polynucleotide or with an expression vector comprising the molecule, which recombinant polynucleotide further comprises an $^{35}S$ promoter from Cauliflower Mosaic Virus, an octopine synthase terminator of *Agrobacterium tumefaciens*, and the regulatory element is the TMV (Tobacco Mosaic Virus) omega translational enhancer element, and having the nucleic acid sequence substantially as denoted by SEQ ID NO: 13 encoding GCD having the amino acid sequence substantially as denoted by SEQ ID NO: 14.(page 14, lines 24-32 of the instant specification).

d. Further evidence of the unintentional, and trivial nature of the omission of a portion of SEQ ID NO: 8 is found throughout the EXAMPLES section of the specification:

i) Expression of the claimed recombinant human glucocerebrosidase is directed by an expression construct including the known full length human glucocerebrosidase coding sequence from ATCC Accession No. 65696 (see Example 1, page 37).

ii) The expressed recombinant human glucocerebrosidase was positively identified as glucocerebrosidase ***of correct molecular size and antigenicity*** by Western blotting (see Example 2, pages 38-40, and Figure 2).

iii) The expressed recombinant human glucocerebrosidase when purified, demonstrated ***complete catalytic activity*** comparable to or better than control human glucocerebrosidase (see Example 3, pages 40-43, Figures 3A-3C and 4A-4C).

iv) The expressed recombinant human glucocerebrosidase, when purified, demonstrated ***binding and uptake by human macrophages*** (biological activity) comparable to or better than control human glucocerebrosidase (see Example 3, pages 40-43, Figures 5A-5D).

Thus, Applicant submits that, according to strict structural and functional assessment of the recombinant human glucocerebrosidase expressed according to the claimed invention (e.g. including the polypeptide having amino acid sequence of SEQ ID NO: 8), SEQ ID NO: 8 relates to the full length amino acid sequence of human glucocerebrosidase, as encoded by SEQ ID NO: 7, and not the truncated polypeptide regretfully included in the sequence identifier listing as filed. Applicant has now provided an amended SEQ ID listing, in which SEQ ID NO: 8 has been amended to include the full length human glucocerebrosidase amino acid sequence in place of the mistakenly truncated listing as filed. Applicant submits that, in view of the arguments and evidence provided herewith, the amended SEQ ID NO: 8 does not constitute introduction of new matter.

***Drawings***

Regarding Fig. 3C- In order to improve definition in Fig. 3C, Applicant is providing the original color drawing of Fig. 3C. A petition for color drawings, including explanations in accordance with the Examiner's comments, and the appropriate fee as set forth in 37 CFR 1.17(h) are submitted. Applicant respectfully requests acceptance of the petition for color drawings in the indicated figures and withdrawal of the objection to Fig. 3C on the basis of poor quality.

***Claims Objections***

The Examiner has objected to claims 98-102, 104-120, 122-128, 142 and 143. Claims 99, 101, 102, 104, 105, 110-113, 116, 118, 119, 122 and 123 have now been canceled, rendering moot the Examiner's objections thereto. Appropriate correction is provided herewith:

1. Claims 106, 107, 108, 124-125 and 142 have now been amended to recite "vacuolar signal peptide" as recommended by the Examiner.

2. Claims 98 and 115 have now been amended to recite "wherein said human lysosomal protein is linked at its C-terminus to a vacuolar signal peptide and at its N-terminus to an endoplasmic reticulum signal peptide" as recommended by the Examiner.

3. Claims 107, 108, 125 and 126 have now been amended to recite "wherein said vacuolar targeting signal peptide comprises of SEQ ID NO:2" as recommended by the Examiner.

4. Claim 109 has now been amended to recite "wherein said human glucocerebrosidase comprises the amino acid sequence as set forth in SEQ ID NO: 8", as recommended by the Examiner.

5. Claim 120 has now been amended to recite "...protein comprises the amino acid sequence..." as recommended by the Examiner.

6. Claim 142 has now been amended to recite "...human glucocerebrosidase which comprises the amino acid sequence as set forth in SEQ ID NO: 8, wherein said human glucocerebrosidase is linked at its C-terminus to the vacuolar targeting signal peptide as set forth in SEQ ID NO: 2 and at its N-terminus to the endoplasmic reticulum signal peptide as set forth in SEQ ID NO: 1." as recommended by the Examiner.

9. Claim 143 has now been amended to recite "...wherein said protein comprises the amino acid sequence of SEQ ID NO: 14", as recommended by the Examiner.

Thus, Applicant respectfully requests withdrawal of the objections to claims 98-102, 104-120, 122-128, 142 and 143.

***35 U.S.C. § 112, Second Paragraph Rejections***

The Examiner has rejected claims 110-113, 127 and 143 as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the Invention. Claims 110-113 have now been canceled, rendering moot the Examiner's rejection thereof. Claims 127 and 143 have now been amended.

Regarding claim 127: Claim 127 has now been amended to recite:

> 127. The plant cell preparation of claim 115, wherein said human lysosomal protein having at least one xylose residue and at least one exposed mannose residue is the main glycan structure of the lysosomal proteins of said plant cell preparation, as measured by linkage analysis.

Thus, claim 127 now relates to the relative portion of the claimed lysosomal protein, having at least one xylose residue and at least one exposed mannose residue, among the lysosomal proteins of the plant cell fraction. Support for such an amendment is found, *inter alia*, in Example 5 of the instant specification (see page 54, line 26, and Fig. 7).

Regarding claim 143, claim 143 has now been amended to independent form, as recommended, and no longer depends from claim 142.

Thus, Applicant believes to have overcome the rejections of claims 127 and 143 on the basis of 35 USC 112, 2nd paragraph.

***35 U.S.C. § 112, First Paragraph Rejections***

The Examiner has rejected claims 98-102, 104-108, 110-119 and 122-128 under 35 USC § 112 1st Paragraph, as failing to comply with the written description requirement. The Examiner's rejections are respectfully traversed. Claims 99, 101, 102, 104, 105, 110-113, 116, 118, 119, 122 and 123 have now been canceled, rendering moot the Examiner's rejections thereof. Claims 98, 106, 107, 108, 109, 114, 115, 120, 124, 125, 126, 127, 142 and 143 have now been amended.

The Examiner has alleged that the specification, while enabling for the polypeptide of SEQ ID NO:8 having a plant glycosylation pattern, and a pharmaceutical composition comprising the polypeptide, is not enabling for any human lysosomal protein having a plant glycosylation pattern or pharmaceutical composition comprising such. Applicant disagrees.

While traversing the Examiner's rejection, and in order to expedite prosecution in this case, Applicant has chosen to amend independent claims 98 and 115 to include the limitation of the human lysosomal protein being encoded by a nucleic acid sequence as set forth in SEQ ID NO: 7, which encodes the polypeptide of SEQ ID NO:8. Support for such an amendment is provided throughout the instant specification, for example, page 14, lines 24-25 (see *supra*). Inasmuch as the Examiner has acknowledged that the specification is enabling for SEQ ID NO: 8, Applicant respectfully requests withdrawal of the 112, 1st paragraph rejection of claims 98 and 115, and claims dependent therefrom.

***35 U.S.C. § 102 Rejections: Garger et al. (US Patent Application No:09/993059).***

The Examiner has rejected claims 98-102, 110-119, 127 and 128 under 35 USC 102(b) as allegedly being anticipated by Garger et al. The Examiner's rejections are respectfully traversed. Claims 99, 101, 102, 104, 105, 110-113, 116, 118, 119, 122, and 123 have now been canceled, rendering moot the Examiner's rejections thereof. Claims 98, 106, 108, 107, 109, 114, 115, 120, 124, 125, 126, 127, 142 and 143 have now been amended.

The Examiner has alleged that Garger et al teaches the recombinant protein of human glucocerebrosidase in transgenic tobacco plants, and the enzymatic removal of sialic acid, galactose and N-acetylglucosamine residues, having xylose and fucose residues and therefore exposed mannose residues. Applicant disagrees.

Garger et al. teaches the transformation and selection of whole transgenic plants expressing and secreting an ER targeted recombinant human lysosomal protein into the intercellular space:

> [0098] The main goal in selecting plants for expression of this protein is the potential for a radical reduction in costs. For the RNA-viral mediated synthesis of rGal-A and rGCB in plants, this is very likely to be achieved through the synergistic combination of three factors:
> [0099] Complex crude extracts from various eukaryote cell production systems may be replaced with a plant leaf homogenate or IF fractions highly enriched in recombinant product.
> [0100] Large-scale, sterile, cell fermentation systems and associated media, capitalization, and waste costs may be replaced with plant biomass. Production is then inexpensively scaled to the quantities desired.

Garger et al. fails to teach production in plant cell culture, or vacuolar targeting of the recombinant protein of human glucocerebrosidase, and does not demonstrate macrophage binding or uptake of the expressed lysosomal protein, thus, the lysosomal protein of Garger et al. does not, and cannot anticipate the claimed recombinant human lysosomal protein, which, due to the pattern of glycoside modification in the vacuole and endoplasmic reticulum possesses a demonstrated capacity for binding and uptake to target cells.

Yet further, inasmuch as the recombinant tobacco plants of Garger et al. are specifically designed to secrete the expressed lysosomal proteins into the intercellular space, Garger et al. is counter-intuitive to, and teaches away from the vacuole-targeted recombinant lysosomal proteins of the instant invention as claimed.

While traversing the Examiner's rejections, and in order to expedite prosecution thereof, Applicant has chosen to amend independent claims 98 and 115 to include the limitations of the human lysosomal protein being contiguously linked at its C terminus to a vacuolar targeting signal peptide and at its N-terminus to an N-terminal endoplasmic reticulum signal peptide.

Thus, Garger et al. does not, and cannot anticipate the human lysosomal protein of claim 98 and the plant cell preparation of claim 115, and claims dependent therefrom.

Support for such amendments is found throughout the instant specification, for example, in now canceled claims 104 and 105.

***35 U.S.C. § 103 Rejections: Garger et al. (US Patent Application No:09/993059), in view of Boller et al (US 6054637) and Stomp et al (6815184).***

The Examiner has rejected claims 104-107 and 122-125 under 35 USC 103(a) as allegedly being unpatentable over Garger et al., in view of Boller et al and Stomp et al. The Examiner's rejections are respectfully traversed. Claims 104, 105, 122 and 123 have now been canceled, rendering moot the Examiner's rejections thereof. Claims 98, 106, 107, 124 and 125 have now been amended.

While noting that Garger et al. fails to teach recombinant human lysosomal proteins linked at the N-terminus to an endoplasmic reticulum signal peptide and linked at the C-terminus to a vacuolar targeting signal peptide, the Examiner has alleged that Boller et al. teaches several signal peptides for vacuolar sorting, and adding vacuolar sorting signal sequences to the 3' end of desirable expressible DNA. Thus, the Examiner alleges that one of ordinary skill in the art would be motivated to combine Garger et al. with Boller et al. to make a human lysosomal fusion protein comprising an ER signal peptide and a vacuolar targeting peptide. Applicant disagrees.

Claims 98 and 115, from which claims 106, 107, and 124, 125 and 126 depend, have been amended as detailed hereinabove. The claimed human recombinant lysosomal protein comprises an amino acid sequence of human glucocerebrosidase, linked to an N-terminus ER signal peptide and a C-terminus vacuolar targeting signal, resulting in a vacuolar-targeted, plant derived human lysosomal protein having exposed mannose residues and exceptional biological activity. In contrast, Garger et al. teaches the secretion of the recombinant human

lysosomal proteins by tobacco leaf cells of whole transgenic plants, by direction of the recombinant polypeptide to the endoplasmic reticulum using an N-terminal ER signal peptide:

> "Using a viral transfection system and transgenic plants, we have expressed enzymes in plants that have potential as therapeutic agents for humans with the metabolic storage disorders known as Fabry disease and Gaucher disease. High specific activity recombinant enzymes were secreted by tobacco leaf cells via a default pathway of protein sorting into the apoplastic compartment, a network of extracellular space, cell wall matrix materials and intercellular fluid (IF). We further developed a novel bioprocessing method to purify these enzymes from the IF fraction."(Garger et al, [0040]).

Thus, Garger et al. not only fails to teach the vacuolar targeting of recombinant human lysosomal proteins in plant cell culture, but teaches away from vacuolar targeting of recombinant human glucocerebrosidase.

Boller et al. do not teach the expression of mammalian or human polypeptides in plant cells, but rather relates to methods for expressing plant proteins naturally occuring the vacuole, having recombinantly removed or inactivated vacuolar targeting signal peptides:

> 1. A process to discharge into the extrallular space of a plant a protein that ***naturally*** has a vacuolar targeting sequence at its C-terminal end which is lost as the protein matures and that is normally directed into the plant vacuole, which process essentially comprises: (a) isolating a DNA sequence coding for said protein; (b) removing from the open reading frame the sequence coding for said vacuolar targeting sequence to form a DNA sequence encoding the mature protein devoid of said vacuolar targeting sequence; (c) splicing the DNA sequence from step (b) into a suitable plant expression vector; (d) transforming the product of step (c) into said plant; and (e) culturing said plant under conditions whereby the protein encoded by the DNA sequence from step (b) is expressed and secreted.
> 2. A process for the production of transformed plant material comprising a gene product that ***naturally*** has a vacuolar targeting sequence at its C-terminal end which is lost as the gene product matures that is secreted into the extracellular space, which process comprises: (a) isolating a DNA sequence coding for a protein that has a vacuolar targeting sequence at its C-terminal end and that is normally directed into the plant vacuole; (b) removing from said DNA

> sequence the sequence coding for said vacuolar targeting sequence to form a DNA sequence encoding the mature protein devoid of said vacuolar targeting sequence; (c) splicing the DNA sequence from step (b) into a suitable plant expression vector; (d) transforming the product of step (c) into a plant; (e) culturing said plant under conditions whereby the protein encoded by the DNA sequence from step (b) is expressed and secreted; and; (f) screening the plant material so treated and isolating positive transformants.
> 3. A recombinant DNA molecule that comprises a structural gene that is in operable linkage with expression signals active in plant cells and codes for a gene product present ***naturally*** in the vacuole and in which a 3'-terminal targeting sequence, which is ***naturally*** present in the gene, has been deleted or otherwise inactivated and that therefore, on transformation into a plant host, produces an expression product that does not contain a functional C-terminal signal sequence and is secreted into the extracellular space of the plant.
> 5. A recombinant polynucleotide comprising in a 5' to 3' direction of transcription: a promoter that is functional in plants and which is operably joined to an open reading frame encoding a vacuolar tobacco basic chitinase that has been modified to target said vacuolar tobacco basic chitinase to the extracellular space by creating a translation stop codon in said open reading frame at the 3' end which results in deletion of the C-terminal amino acids of the vacuolar tobacco basic chitinase necessary for vacuolar targeting; and a transcription termination regulatory region operably joined to said modified open reading frame.
> 7. A recombinant DNA molecule comprising: an open reading frame encoding a protein which is ***naturally*** occurring in a plant vacuole wherein said open reading frame has been modified at the 3' end region necessary for vacuolar targeting to produce a modified open reading frame, wherein said modified open reading frame encodes a modified protein which is targeted to the extracellular space.

Yet further, Boller et al clearly teach the targeting of plant expressed polypeptides to the vacuole as a means for <u>preventing</u> secretion into the intercellular space, and deletion of the vacuolar targeting signal sequence as a means for targeting expression products to the intercellular or extracellular space (see, for example, Boller et al., column 18, line 53, to column 19, line 4):

> "In a second variant, the C-terminal extension of a basic chitinase gene...is removed...or at least inactivated. ***As a result, the gene product is secreted into the intercellular space...***"

> "A further aspect of the present invention...relates to recombinant DNA molecules...in which the 3' terminal targeting sequence...has been deleted or otherwise removed. On transformation into a plant host, ***these constructs produce an expression product that does not contain a functional C-terminal signal sequence and is therefore secreted into the extracellular space.***"

As such, Boller et al. cannot correct the deficiencies of Garger et al. Thus, Applicant submits that neither Garger et al. nor Boller et al. provide motivation for combining the secreted lysosomal protein as taught in Garger et al. with the vacuolar targeting signal for use with naturally occurring plant proteins as taught by Boller et al. to make a fusion protein comprising an N-terminus ER signal peptide and a C-terminus vacuolar targeting signal for expression in plant cell culture, as claimed. Indeed, the combination of vacuolar targeting and an N-terminus ER signal peptide is clearly counter-intuitive to Boller et al.

Stomp et al. teaches the transformation of duckweed plants with expressible polynucleotides modified for secretion of the gene products by addition of an ER targeting signal:

> "These methods comprise...
> (2) Culturing a stably transformed duckweed plant or duckweed nodule culture that expresses at least one biologically active polypeptide comprising a signal sequence that directs secretion of the polypeptide into the culture medium..."(Stomp et al., column 6, lines 51-55); and
> "C: Signal peptides
> Signal peptide that directs secretion of the recombinant protein into the culture medium."(see column 13, lines 5-59)

Thus, Stomp et al., similarly to Garger et al. and Boller et al., is counterintuitive to the use of C-terminal signal sequences for vacuolar targeting of recombinant proteins expressed in plant cells.

Applicant submits that one of ordinary skill in the art would not be motivated to combine the methods for producing secreted recombinant proteins in whole plants by ER targeting, as taught by Garger et al. and Stomp et al., with the methods for expressing plant proteins naturally occuring in the vacuole, having recombinantly added vacuolar targeting signal peptides, as taught by Boller et al. Further, one in possession of the methods for producing the secreted human lysosomal protein as taught by Garger et al., would not be capable of producing the secreted human lysosomal protein of Garger et al. using the vacuolar targeting of Boller et al. and/or

the ER targeting of Stomp et al. Thus, contrary to the Examiner's allegations, Stomp et al. alone, or in combination with Garger et al. and/or Boller et al. cannot remedy the deficiencies of Garger et al., and do not motivate expression of a recombinant human lysosomal protein having both N-terminal endoplasmic reticulum targeting signal peptide AND C-terminal vacuolar targeting signal peptide in plant cell culture.

Thus, Applicant submits that the Examiner has failed to provide evidence that the claimed invention is *prima facie* obvious over Garger et al. alone or in view of Boller et al. and/or Stomp et al. Withdrawal of the 103 rejection is respectfully requested.

***Double Patenting***

The Examiner has rejected claims 98-102, 104-113, 115-120 and 122-128 under 35 USC 101 on the grounds of provisional double patenting as claiming the same invention as claims 27-30, 32, 34-39, 41-44, 46-49, 51-52 and 55-61 of co-pending US Patent Application No. 11/790991.

The Examiner has also rejected claims 114 and 142-144 on the ground of non-statutory obviousness type double patenting for being unpatentable over claims 39 and 45 of co-pending US Patent Application No. 11/790991. Claims 99, 101, 102, 104, 105, 110-113, 116, 118, 119, 122 and 123 have now been canceled, rendering moot the Examiner's rejections thereof.

Issues of provisional and obviousness-type double-patenting and the submission of a terminal disclaimer will be further considered with respect to US Patent Application No. 11/790991 upon indication by the Examiner of allowable claims in the present case.

***New Claims***

New claims 145-149 have been added.

Claim 145 is a dependent claim, relating to a pharmaceutical composition comprising the human lysosomal protein of now amended claim 143.

Claims 146 relates to a human lysosomal protein of the present invention, having an amino acid sequence encoded by SEQ ID NO: 7, linked at the C-terminus to a vacuolar targeting signal peptide. New claim 146 is supported throughout the specification, for example, in previously presented claim 104. Thus, no new subject-matter has been added.

Claims 147-148 relate to the biological activity of the human lysosomal protein of claim 146. Such claims are supported throughout the instant specification, for example, original claims 48 and 49, and Figures 5A- 5D.

Claim 149 relates to a human lysosomal protein of the present invention, having an amino acid sequence encoded by SEQ ID NO: 7, linked at the C-terminus to a vacuolar targeting signal peptide. New claim 146 is supported throughout the specification, for example, in Examples 1 and 2.

***Corresponding Patent Applications***

Applicant wishes to make of record the Official Communication issued by the USPTO on September 26, 2008 in co-pending US Patent Application No. 11/790991. All the material references cited in the '991 application have been made of record in the subject application. Applicants believe that they have fully complied with the Federal Circuit court's concerns raised in *McKesson Information Solutions v. Bridge Medical, Inc.* 82 U.S.P.Q.2D (BNA) 1865 (2007).

In view of the foregoing amendments and remarks, pending claims 98, 100, 106, 107, 108, 109, 114, 115, 117, 120, 124, 125, 126, 127, 128, 129 and 142-144, and new claims 145-149 are deemed to be allowable. Their favorable reconsideration and allowance is respectfully requested.

Respectfully submitted,

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Date: December 22, 2008

***Enclosures:***

- Petition for Extension of Time (Two Months)
- Petition to Accept Color Drawings
- Additional Claims Transmittal Fee